THE EFFECT OF THE REMOVAL OF PERISARC ON REGENERATION IN TUBULARIA CROCEA

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INTRODUCTION

In 1903 Morgan observed that small pieces of Tubularia stems, which ordinarily regenerated hydranths at both ends, formed single hydranths at only one end when the other end of the stem was set upright in sand. Regardless of whether the distal or the proximal end was placed in the sand the exposed end was the one which formed the hydranth. Barth (1938*b*) extended these observations and showed that merely placing a glass capillary over the cut end of a stem would result in a complete inhibition of regeneration at the covered end. He also observed that when the coenosarc terminated a few millimeters below the level of the perisarc no regeneration occurred. It was suggested that oxygen lack was the probable cause for this inhibition, and that, in the last-mentioned case, the perisarc did not permit sufficient O_2 to reach the coenosarc for regeneration to occur. A logical experiment arising from these observations was one in which the perisarc was removed from a region along the length of the stem in order to expose the coenosarc to sea water. Morgan (1903) found that when long slivers of the stem of Tubularia (both perisarc and coenosarc) were cut out, the portions of the stem adjacent to this cut regenerated in the same way that two separated portions of a stem would regenerate, i.e., a hydranth formed at either side of the cut, with the oral ends facing the cut region. The two hydranths so formed were connected at the oral ends during the initial stages of regeneration but separated after a while. Goetsch (1929) briefly reported that when all cut ends of a stem of *Pennaria* were sealed with wax, the removal of some of the perisarc was sufficient stimulus to cause regeneration at the exposed region. Peebles (1931) attempted to get lateral outgrowths from stems of Tubularia by " wounding the perisarc " but failed to do so. The present work was an attempt to work out in some detail the consequences of exposing the coenosarc in Tubularia. These experiments were performed in a preliminary way in the summer of 1937 (when it was found that exposing the lateral coenosarc did result in hydranth formation, especially if the ends were ligatured) and were repeated and quantitative data gathered in the summer of 1938. I should like, at this point, to thank Professor Barth for his many suggestions which were so helpful throughout the course of these experiments.

METHOD

Essentially the same technique as the one described by Barth (1938*a*) was used for selecting the stems, keeping them, and for performing the necessary calculations. Straight, unbranched stems were selected from colonies of long (8–9 cm.) stems. These were selected for uniformity in thickness, etc. The hydranth plus 3–5 mm. of the stem was removed and the required length of stem was cut from the region immediately proximal to this. The ends were then ligatured as desired and the perisarc was removed. This removal was effected by means of a pair of iridectomy scissors used under a binocular microscope. Ligatured stems were selected at random for the various groups in an experiment. The desired measurements (the area of the exposed coenosarc and the diameter of the stem) were made with an ocular micrometer. The stems were kept on a gauze platform in a dish where aerated sea water was circulating constantly. To insure uniformity of conditions all of the stems involved in any one experiment were kept together in the same dish. The rate of regeneration was obtained by measuring the length of the regenerant at the time it was constricted from the rest of the stem, and employing the time required for this to occur as t_1 in the formula $R = \pi r_2 L/t_1$ (see Barth, 1938*a*) where r is the radius of the stem at the region involved, and L is the length of the primordium. In cases (Fig. 1—1 *C*) where the regenerant was not a complete cylinder the volume was obtained indirectly by employing a bit of rubber tubing as a model for the stem. A sector of the tubing equivalent in size and shape to the regenerant was cut out and weighed. It was found that this was from one-third to one-half the total weight of the tubing. The volume of the actual cylinder of stem was calculated using the longest length of the regenerant as L, and then the necessary one-third or one-half was subtracted from this figure. All of the data presented are averages for the various groups considered. Only stems showing regeneration within 72 hours after the beginning of the experiment were considered in the calculations.

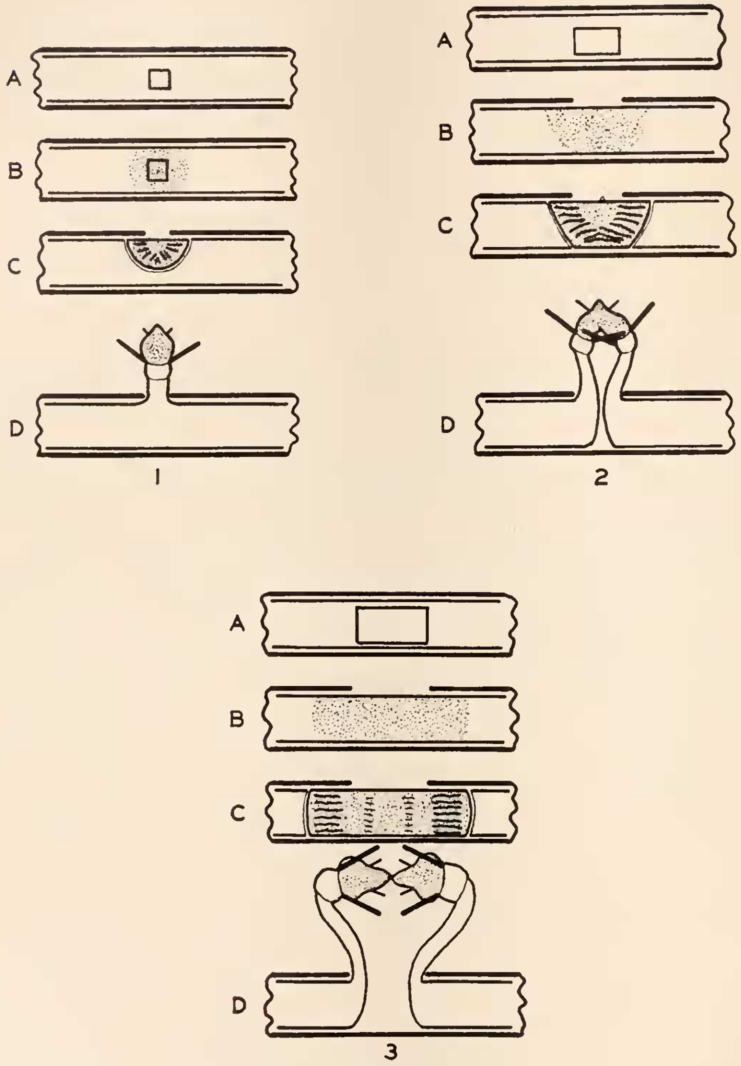


FIG. 1. Diagrams to show the effect of increasing the size of the exposed area upon the morphology of the regenerants. One, 2, and 3 are the three size groups. *A* indicates the amount of perisarc removed. *B* shows the beginning of pigment deposition. *C* shows the constriction of the regenerant from the rest of the stem and the appearance of the tentacle buds. *D* represents the condition of the regenerant after it has emerged.

RELATION BETWEEN THE AREA EXPOSED AND THE RATE OF REGENERATION

After preliminary experiments indicated that the exposure of the coenosarc was a sufficient stimulus for regeneration, an attempt was made to see whether there was any relation between the area exposed and the rate of regeneration. If, as was suspected, exposure to oxygen was the cause for regeneration a greater amount of exposed tissue should result in a larger regenerant. This was found to be the case. Table I presents data from two experiments which illustrate this. Experiment 1 was performed early in June when the stems were thick and experiment 2 was a repetition of the experiment performed in July when the young thin stems prevailed. The results are essentially the same as far as rate of regeneration is concerned. As the area of the exposed region was increased, the rate of regeneration went up. Since the average time in which this regeneration occurred was approximately the same for all of the groups in any one experiment, it can be safely assumed that it was the amount of tissue converted in a unit of time that was increased as more coenosarc was exposed. (See Barth, 1938*b*, where both t_1 and L vary with the oxygen tension.)

TABLE I

Fifteen-millimeter stems. *A*, *B* and *C* are the three size-groups for the areas exposed. *No.*, number of stems used; *Diam.*, diameter in micra; *Area* in square micra; T_1, time in hours; *No. Reg.*, number of regenerants; *Rate*, rate of regeneration.

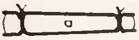
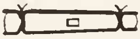
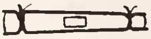
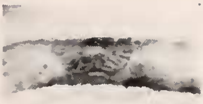
	A	B	C
			
EXP. 1			
NO.	10	9	10
DIAM.	769	789	802
AREA	55073	153793	281559
T_1	43.7	48.1	47
NO. REG.	4	8	9
RATE	36	76	137
EXP. 2			
NO.	10	10	10
DIAM.	457	490	477
AREA	48152	132793	164962
T_1	38.5	38.6	32.4
NO. REG.	7	8	9
RATE	71	89	129

PLATE I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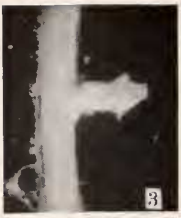
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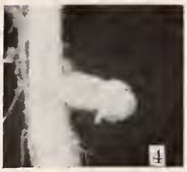
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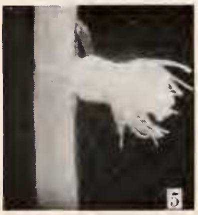
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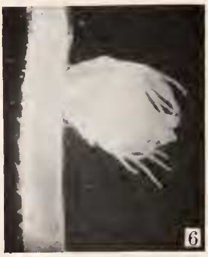
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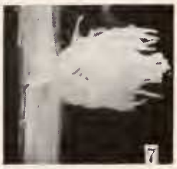
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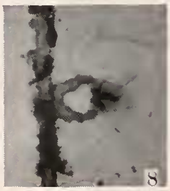
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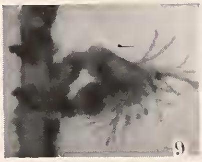
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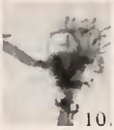
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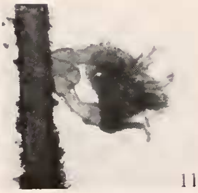
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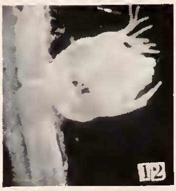
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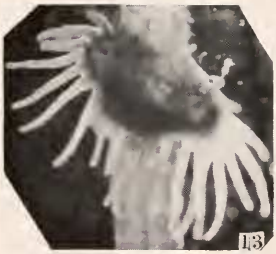
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1. A central regenerant (as in Fig. 1—1*C*) at the constriction stage.
2. A central regenerant (as in Fig. 1—3*C*) before constriction. The tissue is slightly contracted.
3 and 4. Single hydranths (as in Fig. 1—1) after emergence.
5–12. Various degrees of fusion of the two emerged hydranths. (10 was cut from the stem at the point of emergence.)
13. Looking down on two complete hydranths which are connected at the hypostomes.

(Figs. 2 and 13 were photographed by Dr. A. N. Solberg.) (The author wishes to thank Mr. Jack Godrich for his assistance in preparing the graphic material for publication.)

Morphological and Polarity Variations

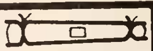
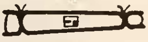
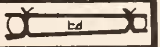
When thicker stems were used (Table I, Experiment 1 and preliminary) in the above experiment it was found that in addition to a variation in the rate of regeneration there was a variation in the morphology and in the polarity of the regenerants which depended roughly on the amount of tissue exposed. As depicted in Fig. 1 and in Plate I, there were three main categories of regenerants. When the exposed area was small (Fig. 1, case 1 ; Pl. I, 1, 3 and 4), only a limited amount of the coenosarc immediately surrounding it was involved in regeneration. In these cases the tissue involved in hydranth formation did not extend completely around the stem but formed a curved disc. When the proximal tentacle buds appeared they radiated out from the exposed central area. The distal tentacles frequently formed during the later stages of regeneration or during emergence. Such regenerants emerged as complete single hydranths whose polarity, i.e., oral-basal axis, was at right angles to the original polarity of the stem. In most of the cases where a greater amount of coenosarc was exposed (Fig. 1, case 2) the tissue involved in regeneration extended completely around the stem but was not symmetrical. A longer portion of the stem was involved in hydranth formation in regions immediately adjacent to the opening, while less and less of the stem was involved the farther it was removed from the opening. Such regenerants formed cylinders with oblique ends and had the appearance of trapezoids when they were viewed from the side (Fig. 1, case 2, *B* and *C*). These emerged as two more or less completely fused hydranths (Pl. I, 5 to 13). The extent of the fusion depended on the length of the region farthest from the opening. Where this was short the two hydranths were completely fused, as this increased in size the bases of the hydranths became separate but the oral portions were in common. All degrees of such fusion were encountered. The oral-basal axis in these cases was at various oblique angles to the original axis. In a few cases very large areas were exposed (the perisarc was removed from about one-half the surface of the stem for a length of 2–3 mm.) and a complete cylinder of coenosarc was involved in hydranth formation. The tentacle buds formed symmetrically around the stem (Fig. 1, case 3, *C*) and when the two hydranths emerged they were complete and mirror-images of each other. They remained fused at the hypostomal region and did not separate even when kept for 10 days. Here the original polarity was retained by the proximal regenerant and was completely reversed in the distal one. These results were similar to Morgan's cases (cited above) except that in his experiments the two hydranths separated. This difference is probably due to a mechanical factor, i.e., in Morgan's cases there was a thin bridge of tissue between the two hydranths and this probably broke very easily when the regenerants contracted, while in the present cases there was a thicker connection which was not broken.

Injury to Tissue

In the light of Child's (1927*b*) experiments on *Corymorpha,* where laceration of the tissue (in this case not covered by a perisarc) was sufficient to cause regeneration, the work described above was open to the valid criticism that injury to the tissue and not exposure to the sea water was the stimulus for regeneration. The following experiment was performed to settle this question: Three groups of stems (Table II) were selected and ligatured at both ends. The perisarc was removed very carefully in the first group and whenever there was any suspicion that coenosarc had been injured the stem was discarded. In the second group the coenosarc was deliberately lacerated after the perisarc had been removed. In the third group a three-sided flap was made in the perisarc and the underlying coenosarc was lacerated after the flap had been deflected. The flap of perisarc was then allowed to fall back into position—covering the lacerated tissue. Table II shows that the first two groups regenerated at approximately the same rates, while the third group did not regenerate at all. This shows conclusively that exposure to sea water is, in itself, a sufficient stimulus for the initiation of regeneration.

Table II

Fifteen-millimeter stems. *A* is the group in which the perisarc was removed without injury to the coenosarc. *B* is the group in which the perisarc was removed and the coenosarc was lacerated. *C* is the group where the coenosarc was lacerated and where the perisarc was replaced. Legend as in Table I.

	A	B	C
			
NO.	9	10	10
DIAM.	725	745	738
AREA	189488	177805	187638
T_1	45.5	47.4	–
NO. REG.	6	5	0
RATE	64.6	67.5	0

Dominance Relations

Since all of the experiments described above were performed on stems which were ligatured at both ends an attempt was made to see whether the open ends of a stem would affect the regeneration of an exposed region of that stem. Three experiments were performed in each of which four groups of 20 mm. stems were set up. In group A both ends were left undisturbed; in group B the distal 2–3 mm. were ligatured so that there was no continuity between that region and the rest of the coenosarc; in group *C* the proximal 2–3 mm. were ligatured; and in group D both ends were ligatured. Perisarc was then removed from the stem at various levels. In Table III the data for an experiment in which the perisarc was removed from a region 5 mm. from the distal end is presented. Table IV contains the data for an experiment in which the perisarc was removed from a region which was 10 mm. from the distal end; and Table V contains the data when the perisarc was removed from a region which was 15 mm. from the distal end. It is apparent, when these data are examined, that the ends, especially the distal end, exert a strong inhibiting influence (dominance) on the central regenerant when these ends are in continuity with the tissue of the stem. When this continuity is broken by a ligature the inhibition is no longer present and the rate of regeneration of the central region increases greatly. The proximal end by itself does not exert nearly as much of an inhibiting influence as does the distal. In Tables IV and V it can be seen that when only the distal end was tied off the rate of regeneration was much higher than when the proximal end was tied off.

Table III

Twenty-millimeter stems. Showing the effects of the ends on a central regenerant. The openings indicated were 5 mm. from the distal ends. *A,* both ends open; *B,* the distal end ligatured; *C,* the proximal end tied off; *D,* both ends ligatured. Legend as in Table I.

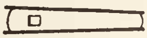
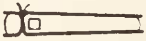
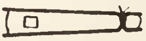
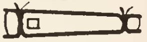
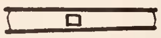
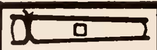
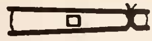
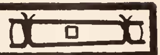
	A	B	C	D
				
NO.	9	9	10	10
DIAM.	659	682	675	708
AREA	170053	145365	157562	192508
T_1	–	36.3	34.3	39
NO. REG.	0	3	3	4
RATE	0	57.7	34.9	115.5

TABLE IV

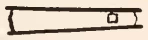
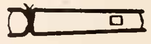
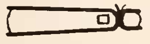
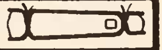
Twenty-millimeter stems. The opening is 10 mm. from the distal end. Rest of table as in Table III.

	A	B	C	D
				
NO.	10	9	10	9
DIAM.	632	586	603	597
AREA	184778	179731	165931	139379
T_1	44	56.6	52	50
NO. REG.	2	5	2	7
RATE	9.1	42.8	8.9	95.6

In fact the distal end exerted just about as much dominance by itself, in these cases, as did both ends combined. *C* in Tables IV and V is as low as *A*. In all three experiments there is a marked increase in the rate of regeneration when the inhibiting effect of both ends is removed. The one group which is in apparent discord with these data is group *C* in Table III. Here, despite the fact that the distal end is not ligatured, the rate of regeneration of the central region is fairly high. This is probably due to the fact that the perisarc was removed from a region which was fairly close to the distal end (appr. 5 mm.). The same factors responsible for the appearance of bipolar regenerants in small pieces of Tubularia are probably responsible for the high rate of regeneration of the central regenerants in this group. This has been explained (Morgan, 1901) as being due to the fact that these tissues are very

TABLE V

Twenty-millimeter stems. The opening is 15 mm. from the distal end. Rest of table as in Table III.

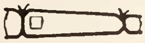
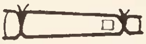
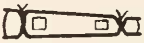
	A	B	C	D
				
NO.	10	10	10	9
DIAM.	553	512	541	514
AREA	142567	117155	161352	111024
T_1	—	44.5	—	47.6
NO. REG.	0	6	0	9
RATE	0	39.3	0	68.9

close together and are probably metabolizing at about the same level and thus do not inhibit each other. Whether or not this is the case, the evidence still indicates definitely that the ligaturing of the ends will result in a great increase in the rate of regeneration of a central exposed region and that the ends do exert a dominance over a central region when they retain continuity with such a region.

Finally, an experiment was performed to determine whether dominance was exerted by a more distally placed exposed region over a more proximally placed exposed region. In Table VI we can see that this is definitely the case. In this experiment 15 mm. stems were used and both ends were ligatured close to the cut surfaces. The two exposed regions were separated by about 10 mm. The distal exposed area, though slightly smaller, completely inhibited the proximal one. Groups *A* and *B* in this table serve as the controls.

Table VI

Fifteen-millimeter stems. Both ends ligatured. *A*, the exposed region at the distal part of the stem; *B*, the exposed region at the proximal end of the stem; *C*, an exposed region at both ends. Legend as in Table I.

	A	B	C	
				
			D	P
NO.	10	10	10	
DIAM.	503	514	521	538
AREA	149021	140021	163738	173309
T_1	42.4	48.6	44.7	—
NO. REG.	5	6	7	0
RATE	41.3	45.9	60.2	0

Discussion

For the first few hours after its exposure the coenosarc of Tubularia is in a condition approximating the early indeterminate stages of an embryo (e.g., the ectoderm of an amphibian embryo). There is a tendency to continue its usual vegetative activity and produce more perisarc to replace that which has been removed. Sooner or later, however, what seems to be a competition between the tendency to retain the vegetative state and the tendency to form hydranth material begins to manifest itself. A visible evidence of this is the appearance of the pink pigment which typifies the first stage in regeneration. Now, depending on the existing conditions (size of the opening, dominance of another region, or inherent conditions within the stem), the coenosarc either becomes definitely determined to form hydranth tissue or retains its original condition. In the former case a hydranth forms and eventually breaks through the thin perisarc which had been secreted in the earlier stages. In the latter the pigment disappears after a while and the coenosarc continues to produce more perisarc until the site of the removal cannot be distinguished from its surroundings. Occasionally, when the opening is very small or when another region is dominating it, the exposed coenosarc does not even begin to regenerate (i.e., no pink pigment is deposited) but continues to form more perisarc as though there had been no interruption. The analogy to amphibian material is made more striking when it is shown that there is a period after which the determination of the hydranth-forming material becomes irreversible. This has been shown by Peebles (1931) and by another method by the author in some unpublished preliminary work. The analogy breaks down, however, when the stimulus for the determination is considered. Although the primary stimulus for form and organ formation in amphibian embryos may come from an outside source, the work of the past few decades has indicated that the immediate cause of determination of a given structure comes from adjacent tissues. In Tubularia, as well as in many other hydroids (Child, 1927*a*) the stimulus for hydranth formation comes from outside of the organism. (The various parts of the hydranth may be determined as in the Amphibia). Many external agents have been cited (Child, 1929) as the stimulators for regeneration.

In referring to the experiments in which stems of Tubularia were either placed end down in sand or in which the end was capped with a glass capillary, both Morgan and Barth suggested that an oxygen lack might be responsible for the ensuing inhibition. It was implied that the presence of sufficient O_2 would allow regeneration to proceed. Accumulated wastes rather than O_2 lack was not excluded as a possible cause of the inhibition. The present experiments do exclude this since the sea water in which the stems were kept was well aerated and was kept in constant circulation (the glass-capping experiments were repeated under these conditions and the same results were obtained). It seems likely, therefore, that the inhibition of regeneration is due to oxygen lack; and that when an adequate supply of O_2 reaches the tissue it serves as a stimulus for regeneration. This idea is rendered more plausible when the effects of varying the oxygen tension on the rate of regeneration are considered. Barth (1938*b*) found that both the length of the regenerant increased and that the time in which constriction of the regenerant occurred decreased as the oxygen tension was increased. This indicates that as the only variable in these experiments, i.e., oxygen, was increased a greater amount of tissue was converted into hydranth tissue in each unit of time. This bears a definite similarity to the experiments cited above in which the size of the regenerant increased when more coenosarc was exposed to the influence of the sea water and suggests a similar causal relationship. The similarity between the action of oxygen and the exposure of the coenosarc is made more apparent by some unpublished work of Dr. J. A. Miller. (Dr. Miller was kind enough to grant the author permission to mention some of his material in relation with the present work.) This work of Miller's concerns the effects obtained with varying the oxygen tension of the sea water in which small bits of Tubularia stem were kept. These small stems ordinarily yield bipolar regenerants. As the tension of the oxygen was increased, the number of incomplete bipolars was increased. This indicates that the various regions of the regenerant increased in size and that the central portion of the small stem was used for more distal structures instead of for the more basal regions which they would have formed in a lower concentration of oxygen. Since this increase in primordia size occurred equally at both ends, there was not enough material to form complete hydranths and two symmetrical incomplete forms resulted—more or less of the basal regions were missing. These results are, to some extent, the converse of the results obtained when the perisarc was removed from the stem. When the area exposed was small the regenerant was small and a single complete hydranth formed,—just as the bipolars were usually small complete hydranths when the oxygen tension was low. When the openings were sufficiently large more tissue was involved and the regenerants were enlarged and were more complete. When Miller increased the oxygen tension the bipolars became more incomplete for the same reason, i.e., the regenerants became enlarged but at the expense of their basal regions. The striking similarity between the effects of exposing the coenosarc and increasing the oxygen tension leads one to conclude that the former is also probably the effect of the oxygen in the sea water. The experiment where the perisarc was replaced helps to substantiate this.

If the premise that an increased oxygen supply stimulates the coenosarc to form hydranth tissue is granted, then the results obtained in these experiments can be explained very readily. The coenosarc must be metabolizing at a certain level before hydranth tissue will form. When a small opening is made in the perisarc only the tissues immediately around the opening get enough O_2 to raise them to this level. Since the basal portions of the hydranth (including the proximal tentacles) form at the lower level of activity, and since this lower level is symmetrical around the opening, a small but complete hydranth forms. But, since the region exposed to the oxygen is at the side, and the high activity level is around this, the new hydranth's polarity is at right angles to the polarity of the stem. The other form and polarity variations may be explained in the same way. As more oxygen is admitted into the stem, more of the coenosarc is activated to form hydranth tissue and, depending on the resulting gradient of oxygen, the two hydranths produced are more or less fused. If a small region of the stem opposite the opening is activated the basal regions as well as the distal will be fused. If a greater region of this part of the stem is activated the basal portions of the hydranth will be separate. And as this region increases in length more and more of the hydranths become separated until two complete forms are produced.

The work on dominance does not throw any new light on the factors involved in this phenomenon, but simply illustrates the inhibition exerted by the ends upon a central region.

SUMMARY

1. Experiments in which lateral regions of the coenosarc of Tubularia were exposed indicate that the rate of regeneration in such regions is proportional to the area exposed to the influence of freely circulating sea water.

2. The morphology and polarity of central regenerants in thicker stems varies with the area exposed. Forms ranging from single complete hydranths, whose polarity was at right angles to the original polarity, to two hydranths, fused to a greater or lesser extent, were obtained. The polarity of the double hydranths varied with the degree of fusion. All angles to the original polarity were obtained.

3. Injury to the exposed coenosarc is not a factor in the present cases.

4. The ends of a stem exert a definite dominance over the exposed central regions. This dominance may be removed by ligaturing the ends. When this is done the rate of regeneration of the central region increases greatly.

5. A more distally placed central exposed region inhibits another exposed region placed more proximally on the same stem.

6. It is suggested that the probable stimulator of regeneration in Tubularia is the oxygen in the sea water. The results on rate of regeneration and on morphological and polarity variations may be explained by the amount of oxygen available to the coenosarc when areas of different size are exposed.

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